REMARKS

The instant application was filed on June 1, 2001, claiming priority to U.S. Provisional Application No. 60/209,139, filed on June 2, 2000. The instant application was filed with 35 claims, all of which were rejected in the Office Action under reply.

With the present amendments, claim 2 has been canceled and its subject matter has been incorporated into independent claim 1. With the cancellation of claim 2, claims 14-16 have been amended to depend from claim 1 rather than claim 2. In addition to the foregoing, claims 27 and 28 have been amended to recite additional steps relating to the bDNA *in situ* hybridization techniques of the claimed invention.

The rejections set forth in the Office Action are addressed in part by the claim amendments set forth herein and are otherwise traversed for the reasons that follow.

CLAIM REJECTION – 35 U.S.C. § 102(e)

Claims 1, 4, 5, 11, 13, 20, 21, 24, and 26 stand rejected under 35 U.S.C. § 102(e) as anticipated by Sarto et al. (U.S. Patent No. 6,022,689). This rejection is respectfully traversed.

The Sarto et al. reference relates to a fluorescence *in situ* hybridization technique wherein a nucleic acid probe is subjected to the following steps: (i) the nucleic acid probe is added to a slide; (ii) the slide is dried to remove excess moisture; (iii) a target nucleic acid is added to the slide followed by a hybridization solution; (iv) the contents of the slide are denatured and hybridized; and (v) a post-hybridization wash of the slide is performed.

The Sarto et al. reference does ***not*** describe the bDNA procedure of amended independent claim 1. Accordingly, claims 1, 4, 5, 11, 13, 20, 21, 24, and 26 are not anticipated by Sarto et al. Because Sarto et al. does not anticipate the referenced claims, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 102(a)

Claims 1-4, 6, 9-23, and 27-33 stand rejected under 35 U.S.C. § 102(a) as anticipated by Antae et al. This rejection is respectfully traversed.

The Antao et al. reference is a paper by inventors Vincent P. Antao and Audrey N. Player and non-inventor Janice A. Kolberg. The reference describes the use of the *in situ* bDNA assay for RNA and DNA targets in individual cells or tissue sections (page 83). The references also describes the incorporation of non-natural nucleotides in the signal amplification system to reduce nonspecific

hybridization since the non-natural nucleotides will not base pair with the natural sequences that are present in the signal amplification system (page 82).

Contrary to the Examiner's position, Antao et al. does ***not*** disclose step 1(c) wherein the biological material is washed with a detergent at a temperature in the range of approximately 21°C to 60°C. At page 86 of Antao et al., "wash buffer 2" is used as the post-hybridization buffer to wash the analyte-target probe complex; Antao et al. provides no temperature for the post-hybridization washing step. As noted on page 84, wash buffer 2 consists of 0.1x SSC (sodium chloride/sodium citrate – a buffer) and 1mM EDTA (ethylenediamine tetraacetic acid – a preservative); both ingredients are clearly not detergents. Unlike wash buffer 2, wash buffer 3 does use a detergent, specifically, 0.1% Brij-35 detergent. Wash buffer 3, however, is only used for hybridization of the alkaline phosphatase label probe; like wash buffer 2, it is not used to wash the biological material after hybridization.

Because Antao et al. does not teach or suggest washing the biological material in a washing fluid comprised of a detergent at a temperature in the range of 21°C to 60°C, it follows that Antao et al. does not anticipate claims 1-4, 6, 9-23, and 27-33. Accordingly, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 103(a)

Claims 1-4, 11-13, 16, 17, and 20-27 stand rejected under 35 U.S.C. § 103(a) as obvious over Schaeren-Wiemers et al. in view of Nolte and Decimo. This rejection is respectfully traversed.

Shaerens-Wiemers et al. relates to a non-radioactive *in situ* hybridization assay with digoxigenin ("DIG")-labeled cRNA probes for localization of selected mRNA species in tissue sections and cultured cells from the central nervous system.

As the Examiner notes, Shaerens-Wiemers et al. does ***not*** teach: (i) using bDNA hybridization detection means for an *in situ* hybridization assay; (ii) proteinase K for permeabilizing; or (iii) washing at temperatures of 21°C to 69°C. Accordingly, as a primary reference, Shaerens-Wiemers et al. does not render obvious the claimed invention.

As a secondary reference, the Examiner cites Figure 1 of Nolte for the features of claim 2 (Office Action, page 7) and Decimo et al. for the features of claim 1, step (c) (Office Action, page 8).

The Nolte reference is a chapter in a book that outlines how the bDNA signal amplification system works. As noted by the Examiner, Nolte does ***not*** teach the application of the bDNA procedure for *in situ* hybridization, but suggests that bDNA may have application in *in situ* hybridization on page 231 (*see also,* Office Action, page 8). The discussion from Nolte that the Examiner identifies is a very brief suggestion in the Summary at the end of the reference that discussion possible future applications of

in situ hybridization. Within this very brief suggestion, Nolte provides no teachings whatsoever of specific hybridization steps that could be used for such bDNA hybridization.

The Decimo et al. reference describes *in situ* hybridization of nucleic acid probes to cellular RNA. The Decimo et al. reference does ***not*** use bDNA hybridization. Further, the post-hybridization washing step of Decimo et al. does not teach or suggest the use of a detergent (*see*, Decimo et al., pp.193-196, Protocols 5 and 6). Accordingly, Decimo et al. fails to correct the deficiencies of Schaeren-Wiemers et al. and Nolte with respect to the post-hybridization washing step of claim 1(c) as well as the bDNA hybridization steps set forth in amended claim 1.

Because Schaerens-Wiemers et al. in view of Nolte and Decimo et al. does not disclose the post-hybridization washing step of claim 1(c) nor the hybridization steps for bDNA *in situ* hybridization as set forth in amended claim 1, it follows that the hypothetical combination of Schaerens-Wiemers et al. in view of Nolte and Decimo et al. does not render obvious claims 1-4, 11-13, 16, 17, and 20-27. Accordingly, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 103(a)

Claims 6-10 stand rejected under 35 U.S.C. § 103(a) as obvious over Schaeren-Wiemers et al. in view of Nolte and Decimo et al. as applied to claim 1-4 above and further in view of Xu. This rejection is respectfully traversed.

The Schaeren-Wiemers et al. in view of Nolte and Decimo et al. references are discussed immediately above.

The Xu et al. reference describes *in situ* hybridization of mRNA with hapten labeled probes such as DIG, fluorescein, or biotin. The Examiner cites Xu for the teachings of probes of 0.1 to 0.5 μg; washing solutions having Triton-X-100, NaCl, and KCl; and *in situ* probes having 32-36 nucleotides in length (*see*, Xu et al., pp.94 and 95; Office Action, p.10).

In view of the discussion set forth above that Schaeren-Wiemers et al. in view of Nolte and Decimo et al. does not render obvious claims 1-4, it follows that the additional teachings of the Xu reference will not serve to render obvious claim 6-10, which depend from claim 1. In light of the foregoing, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 103(a)

Claim 5 stands rejected under 35 U.S.C. § 103(a) as obvious over Schaeren-Wiemers et al. in view of Nolte and Decimo and Xu and further in view of Sarto et al. This rejection is respectfully traversed.

The Schaeren-Wiemers et al. in view of Nolte, Decimo et al., and Xu references are discussed in the previous two obviousness rejection responses. The Sarto et al. reference was discussed in the response to the Examiner's first anticipation rejection. Within the context of this rejection, the Examiner cites Sarto et al. for the teaching of 5 to 20 μg/mL of proteinase K for permeabilizing cells prior to *in situ* hybridization.

In view of the discussion set forth above that Schaeren-Wiemers et al. in view of Nolte and Decimo et al., does not render obvious claims 1-4, it follows that the additional teachings of the Sarto et al. reference will not serve to render obvious claim 5, which depends from claim 1. With respect to the Xu reference, the Examiner only applied Xu to claims 6-10 as set forth above and does not specify how the Xu reference applies to claim 5. Notwithstanding the confusion with respect to the Xu reference, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 103(a)

Claims 14 and 15 stand rejected under 35 U.S.C. § 103(a) as obvious over Schaeren-Wiemers et al. in view of Nolte and Decimo and further in view of Kern et al. This rejection is respectfully traversed.

The Schaeren-Wiemers et al. in view of Nolte and Decimo et al. references are discussed above. The Kern et al. reference is a Chiron Corporation paper outlining the bDNA assay procedure for quantification of HIV-1 RNA in plasma. The Examiner cites Kern et al. for the disclosure of 0.7 fmol of preamplifier per μL and 1.0 fmol of bDNA amplifer per μL (*see*, Kern et al., p.3197, col.2, last ¶; Office Action, p.13).

In view of the discussion set forth above that Schaeren-Wiemers et al. in view of Nolte and Decimo et al., does not render obvious claims 1-4, it follows that the additional teachings of the Kern et al. reference will not serve to render obvious claims 14 and 15, which originally depended from claim 1 and are now dependent on claim 1 (claim 2 having been canceled when its subject matter was incorporated into claim 1). In light of the foregoing, applicants respectfully request reconsideration and withdrawal of this rejection.

CLAIM REJECTION – 35 U.S.C. § 103(a)

Claims 28-35 stand rejected under 35 U.S.C. § 103(a) as obvious over Siadat-Pajouh in view of Nolte. This rejection is respectfully traversed.

Siadat-Pajouh et al. relates to a fluorescence *in situ* hybridization technique to detect one to five copies of the human papillomavirus ("HPV") genome using DIG-labeled oligonucleotides. In the Office

Action, the Examiner acknowledges that Siadat-Pajouh does ***not*** teach using bDNA for signal amplification and detection of nucleic acid analytes *in situ* (Office Action, p.16).

The Examiner cites Nolte for the teaching of bDNA *in situ* hybridization. As noted above in the discussion for the Schaeren-Wiemers et al. in view of in view of Nolte and Decimo et al. references, the Nolte reference does ***not*** disclose any specific procedures for the bDNA *in situ* hybridization. Given the lack of procedural guidance from Nolte, it follows that the hypothetical combination of Siadat-Pajouh in view of Nolte et al. will not lead the ordinary artisan to the invention as recited in claims 28-35. Accordingly, applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

As each of the Examiner's rejections have been addressed and overcome with this paper, applicants respectfully request reconsideration and withdrawal of all claim rejections and passage of this application to issue.

Should the Examiner have any questions concerning this response, she is welcome to telephone the undersigned attorney at (650) 330-4913 or at canaan@reedpatent.com.

Respectfully submitted,

By: 
Karen Canaan
Registration No. 42,382

REED & EBERLE LLP
800 Menlo Avenue, Suite 210
Menlo Park, California 94025
(650) 330-0900 Telephone
(650) 330-0980 Facsimile